Analysis of Diatom Blooms Using DNA Fingerprints

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LONG-TERM GOALS

My long-term goals are to understand the interaction between the biological and environmental factors that dictate the timing and magnitude of diatom blooms. I am particularly interested in how species behavior is coupled to environmental conditions and how the extent of genetic and physiological diversity within a population influences its future ability to bloom.

OBJECTIVES

The premise that guides my research is that phytoplankton community dynamics reflect a complicated interaction between environmental variability and the inherent genetic and physiological variation present within individual species of phytoplankton. My goal is to determine how genetic and physiological diversity is maintained within individual species of diatoms and how this diversity is shaped by different environments and on different time scales. Ultimately, this should allow me to determine how population diversity is coupled to future blooming capabilities.

APPROACH

The goal of this research is to determine how genetic and physiological diversity is shaped by the environment in order to better understand diatom bloom dynamics. Our studies focus on the unicellular centric diatom *Ditylum brightwellii* because of the importance of this diatom in coastal waters and because of the ease of identifying it in mixed populations. We examine genetic diversity within diatom populations by utilizing high-throughput DNA fingerprinting techniques. We base our DNA fingerprints on highly repetitive regions of DNA known as microsatellites. The length of any given repetitive region can vary dramatically between individuals and so can be used to define individuals. We determine genetic diversity within *D. brightwellii* populations by first isolating individual cells into about 1 ml of media (in a 48-well plate) and then allowing the cells to divide asexually (1-2 weeks). Each isolate can be thought of as a different individual composed of many genetically identical cells. A subset of isolates is maintained in culture for physiological studies. DNA is extracted from all isolates and polymerase chain reaction (PCR) is used to amplify specific microsatellites. The length of a microsatellite defines the allele size; the composite of allele sizes at different microsatellite loci defines the DNA fingerprint of an individual; the combination of individual fingerprints defines a population. We use the population-based information to determine whether genetically defined populations co-vary with different environmental conditions.

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WORK COMPLETED

Over the past year, we have finalized and published our work on analysis of a field study that followed changes in genetic diversity over the course of a spring bloom. The manuscript resulting from this work is entitled "Maintenance of clonal diversity during a spring bloom of the centric diatom *Ditylum brightwellii"* and was published in Molecular Ecology (Mol. Ecol. (2005) **14**, 1631–1640). A second manuscript entitled "Spring bloom development, genetic variation, and population succession in the planktonic diatom *Ditylum brightwellii*" has been submitted and two more manuscripts are in preparation.

RESULTS

The work described below has been conducted by me, my postdoc Tatiana Rynearson, two undergraduates Tasha Snow and Wendy Guo, my technician, Ellen Lin, physical oceanographer Mitsuhiro Kawase and his technician Christian Sarason.

The first study completed over the past year focused on bloom dynamics of *D. brightwellii*. We sampled a *D. brightwellii* bloom over the course of 11 days when cell number for this species increased seven fold. The genotypes at three microsatellite loci were determined for 607 single-cell isolates. Genetic diversity remained high during the bloom and 87% of the cells sampled each day were genetically distinct. No significant differences in microsatellite allele frequencies were observed among daily samples indicating that the bloom was comprised of a single population. No sexual stages were observed, although linkage equilibrium at two loci, high levels of allelic and genotypic diversity, and heterozygote deficiencies were all indicative of past sexual reproduction events.

One of the more fascinating aspects of this study is that we were able to determine whether particular clonal lineages were re-sampled at a higher than expected frequency (Fig. 1). Sixty-nine clonal lineages were sampled two or more times during the bloom, and two clones were sampled seven times. One clonal lineage that was detected four times during the bloom had been previously identified once in November of 1997 in Hood Canal, which suggests that this was a particularly successful clonal lineage that persisted in Hood Canal for at least 2.5 years. Based on the frequency of resampled clonal lineages, capture–recapture statistics were used to determine that at least 2400 genetically distinct clonal lineages comprised the bloom population. This study revealed that the pulse of biomass generated during a bloom is dictated by the response of thousands of clonal lineages.

At the height of the bloom, a windstorm diluted cell numbers by 51% and coincided with a change in the frequency distribution of some resampled lineages. The extensive clonal diversity generated through past sexual reproduction events coupled with frequent environmental changes appear to prevent individual clonal lineages from becoming numerically dominant, maintaining genetic diversity and the adaptive potential of the population.

Two distinct conclusions can be drawn from this data set. First, we can detect when particular clonal lineages have a growth advantage over others as evidenced by the repeated sampling of certain lineages. Second, genetic diversity appears to be maintained within bloom populations because wind-storms and other environmental variables reshuffle which clonal lineages possess a growth advantage.

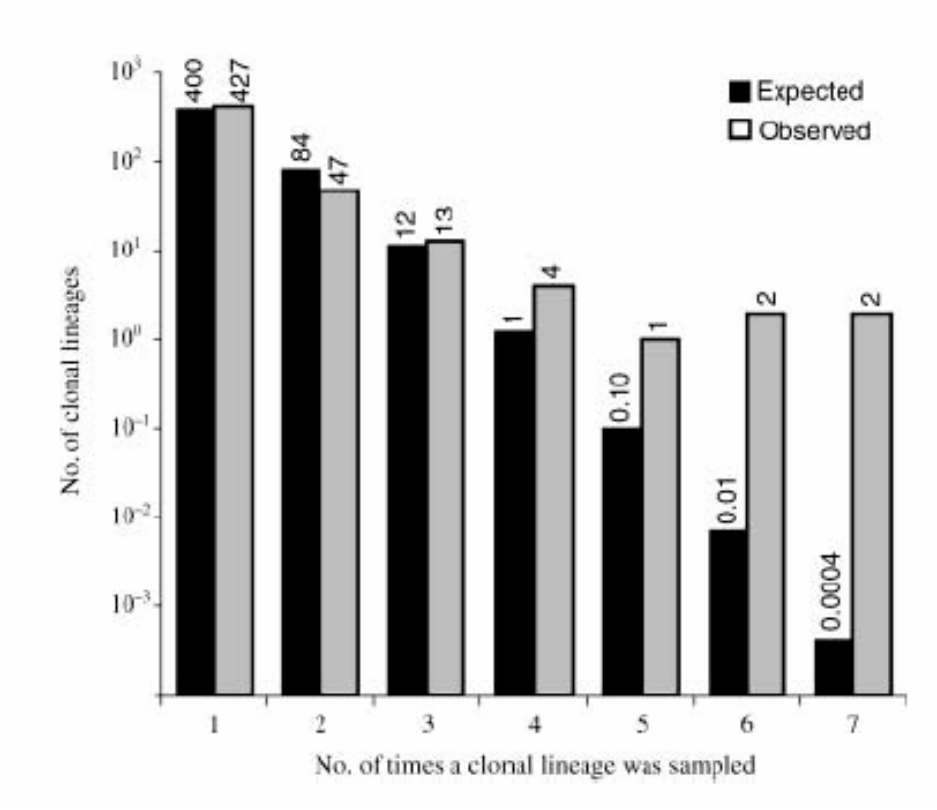

Fig. 1 Expected and observed distributions of sampling frequency of individual clonal lineages. The observed distribution represents the frequency of clonal lineages that were sampled between 1 and 7 times over the course of the bloom. The expected distribution was obtained using a zero-truncated Poisson distribution with $\lambda = 0.418$. Numbers above each bar indicate the number of clonal lineages represented in each category.

The second study was conducted in collaboration with Dr. Jan Newton of the Washington State Department of Ecology. We examined spatial and temporal variation in the genetic composition of *D. brightwellii* during multiple spring blooms. We collected 21 water samples between February and June from five locations in Puget Sound and the Strait of Juan de Fuca, Washington. We analyzed up to three microsatellite loci from 707 individual cells. Two genetically distinct populations were identified during the sampling period. One population, defined as the early population, was detected at all locations from February to April. In May and June, it could no longer be detected. Instead, a second, genetically distinct population, defined as the late population, was present. Both populations formed blooms with abundances of up to 3500 cells·L^{-1} and were characterized by high levels of diversity: on average, 94% of cells in each sample were genetically distinct and gene diversity was 0.74. The 18S and 5.8S rDNA sequences of cells from the two populations were identical and the internal transcribed regions, ITSI and II, contained 1.1% sequence divergence between populations indicating that the two populations were members of the same species.

One of the more interesting aspects of this data set was that we observed significant correlations between environmental conditions and the presence of one or the other population (Fig. 2). The early population was present in samples with relatively high $SiOH_4$ concentrations (41 - 81 µM) and low levels of solar irradiance (1370 - 4449 $W \cdot m_2$). In contrast, the late population dominated under conditions of higher solar irradiance (4371 - 4449 $W \cdot m_2$) and lower $SiOH_4$ concentrations (22 - 42 µM).

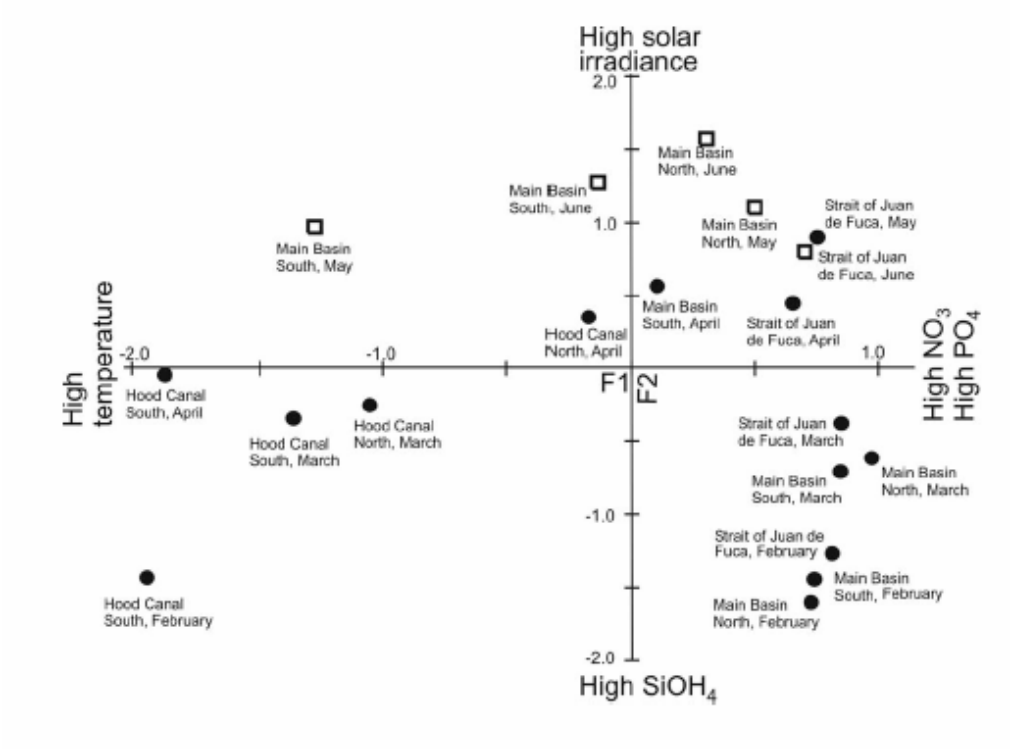

Figure 2. Factor analysis of physical/chemical properties for the water samples. Factor one (F1) is positively correlated with high NO_3/PO_4 concentrations and negatively correlated with high temperature. Factor two (F2) is positively correlated with high solar irradiance and negatively correlated with high $SiOH_4$ concentrations. Filled circles and open squares represent samples that contained early or late populations, respectively.

Our most recent work builds on these studies. We have focused laboratory studies on measuring growth rates of *D. brightwellii* isolates that represent the different populations. We are currently maintaining 24 different isolates in artificial seawater media supplemented with either 10, 100 or 800 µM NO_3 or 10 or 800 µM silicate. We have developed a higher-throughput means of growing and monitoring cultures. We now grow our isolates in 48-well plates and use a plate reader to measure chlorophyll *a* fluorescence from the individual wells. These on-going physiological studies will reveal differences in the physiological potential of cells from different populations.

The second direction our work has taken is to move toward understanding the relation between population succession and water circulation in Puget Sound. We have been collaborating with UW physical oceanographer, Mitsuhiro Kawase, to determine the movement of diatom-sized particles into and throughout the Sound using a numerical model of Puget Sound circulation that is based on the Princeton Ocean Model (Kawase 1998). Runoff, wind speed, bathymetry, and tidal data are all used as

input for the model, which has been run for the period overlapping with our monthly sampling in the spring of 2000. Mitsuhiro Kawase and Christian Sarason have developed algorithms to incorporate the movement of diatoms in surface waters into those model runs (Fig. 3). For example, using this model, we have determined that surface currents are unable to flush the early population from Puget Sound between April and May, the time period when the early population disappears from Puget Sound and the late population appears. These results indicate that either biological processes such as natural selection or physical processes such as upwelling may dictate the succession of populations. Future simulations will incorporate growth and replication of diatoms during the model runs as well as the movement of diatoms at depth.

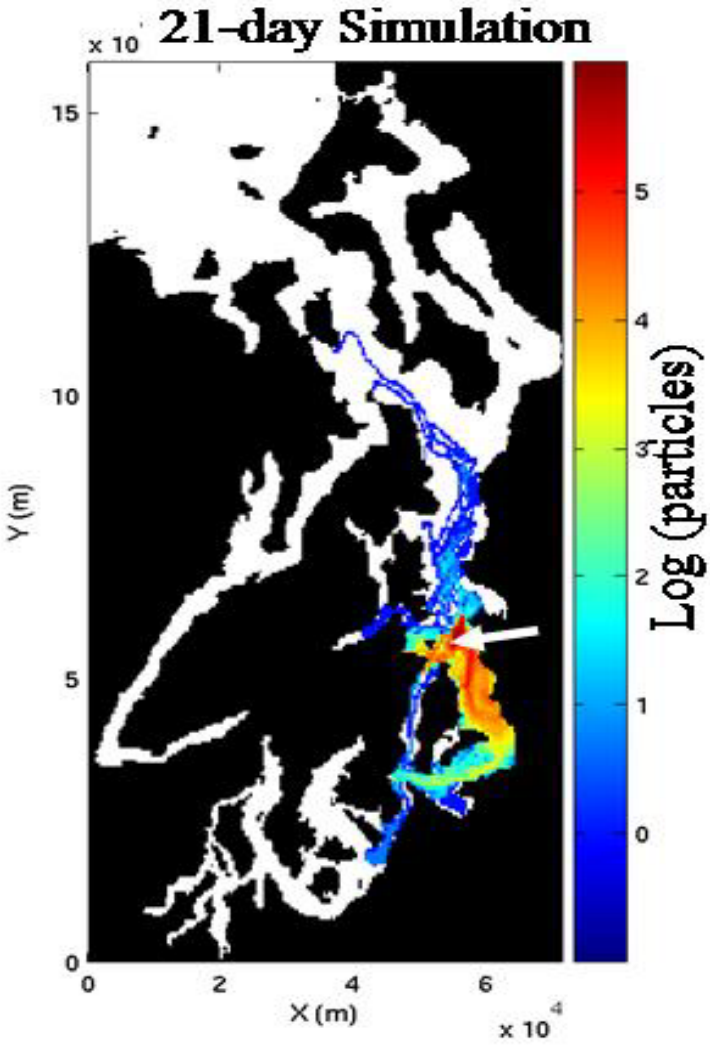

Figure 3. Simulation of diatom movement in Puget Sound surface waters over a 21-day period. Five hundred diatom "particles" were released over the course of a tidal cycle from the location indicated by the white arrow. Each track represents the movement of one diatom. The color bar indicates the density of diatom particles in surface waters. Most particle remain within South Puget Sound during the run.

IMPACT/APPLICATION

We have shown that diatom populations are composed of thousands of genetically distinct individuals and have used population genetics approaches to define genetically distinct populations. By combining genetic techniques with more standard oceanographic measurements, we have been able to identify conditions under which different populations of a single species of diatom will bloom. Ultimately, this work will allow us to examine in more detail which physiological characteristics are under selection. Moreover, we can examine the bloom dynamics of genetically distinct populations under changing environmental conditions. This new way of thinking about physical and genetic partitioning in the marine environment has ramifications for management of coastal regions and investigations of harmful algal blooms.

PUBLICATIONS

Rynearson TA and EV Armbrust (2005) Maintenance of clonal diversity during a spring bloom of the centric diatom *Ditylum brightwellii*. *Molec. Ecol.* **14**, 1631-1640. [published, refereed]

Rynearson, TA, JA Newton and EV Armbrust. (submitted) Spring bloom development, genetic variation and population succession in the planktonic diatom *Ditylum brightwellii*

Rynearson, T.A., E.V. Armbrust, E.V., M. Kawase, and C. Sarason. 2005 Genetics or environment? Regulation of diatom blooms in Puget Sound, WA. ASLO-TOS Ocean Research Conference